colorimetric method for 0.5 ppm trichloramine. Again, the discrepancy between the titration and colorimetric methods is not reasonable.

OXIDIZED MANGANESE. Although Mn(II) does not interfere in any of the methods for chlorine, it can, under slightly alkaline conditions, be oxidized to Mn(IV) by both air and chlorine. Because the pH is 7.0 in the SNORT method, Mn(IV) is not, however, completely reduced to Mn(II).

By using the manganese dioxide colloidal sol prepared above, from 80 to 100% of the stoichiometric amount of blue product was produced. The results were dependent on the age as well as the pH of the sol. The higher the pH, the lower the interference from the sol; however, the results were quite variable.

OTHER INTERFERENCES. In general all strong oxidizing agents interfere with all methods for chlorine in stoichiometric amounts (*4*). Some of these of possible importance include iodine, bromine, chlorine dioxide, and oxidized manganese. The only exception to this is the König, cyanogen chloride method which is a difficult two step extraction procedure.

Up to 1 m*M* Fe(III) solutions were tested and produced no measurable interference at 625 nm, even with a distilled water blank. The higher concentrations of iron, however, did produce a green visual product which would have to be balanced with a blank when using visual comparators. Because of the chlorine demand present in the Fe(III) reagents, these solutions with chlorine were standardized by amperometric titration, the results of which were the basis of comparison.

Nicolson (*4*) found 0.5 ppm Fe(III) gave a 2% interference with acid orthotolidine and a 10% interference in Palin's neutral orthotolidine method (*12*, *13*).

Solutions containing up to 2 m*M* nitrite were tested and produced no positive interference with SNORT. Addition of nitrite solution to SNORT products produced no negative interference. By contrast the interference of nitrite in the acid orthotolidine procedure is well known (*4*, *5*).

Sensitivity and Precision. The molar absorptivity of the 625-nm peak of the SNORT product is 3.41×10^4 liter mole^{-1} cm^{-1} at pH 7.0. This gives the method a sensitivity, that concentration producing 0.001 absorbance per cm, of 0.0021 mg/liter Cl_2. This is slightly less than the 0.0014 mg/liter Cl_2 sensitivity of acid orthotolidine at pH 1 but more sensitive than the 0.0036 mg/liter Cl_2 for DPD quoted by Nicolson (*4*).

The precision of the method was determined from ten aliquots taken from a single solution measured over a two-hour period. The sample mean concentration was 1.412×10^{-5} *M*. The sample standard deviation was 5.5×10^{-8}*M* for a relative standard deviation of 0.39%. The precision of the method remains good up to 0.1 m*M* as the method obeys Beer's law to that concentration using the recommended concentration of orthotolidine.

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Automated Colorimetric Microdetermination of Phenols by Oxidative Coupling with 3-Methyl-2-Benzothiazolinone Hydrazone

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The previously reported oxidative coupling reaction of phenols with 3-methyl-2-benzothiazolinone hydrazone is the basis for a new automated general method for the determination of phenols. Conditions are also given for performing the method manually to compare it with other known methods. The widely used 4-aminoantipyrine method for phenols suffers in that many *p*-substituted phenols are not detected. The present method, however, is less dependent on the position of substituent groups. Furthermore, most of the phenols tested are determined at lower concentrations than are possible by the 4-aminoantipyrine method. Some phenols are determinable even in the parts-per-billion range without prior concentration-extraction into organic solvents. The system operates at a rate of 20 samples per hour alone or when combined with automated steam distillation of the phenols prior to the colorimetric reaction. For monitoring water supplies for phenols, the steam distillation step should usually provide the only cleanup necessary. For other purposes—e.g., determination of pesticide residues which contain hydrolyzable phenol moieties—preliminary sample treatment procedures will be required.

AS A CONSEQUENCE of current efforts to automate general methods for pesticide residue analysis (*1*), a suitable phenol method as a common analytical basis for several types of pesticides has now been developed. Roughly 25% of the pesticides on the world market today are compounds possessing a substituted phenol moiety which may be more or less easily cleaved from the molecule. Such compounds are found among certain herbicides, a few fungicides, numerous carbamates, and several organophosphorus insecticides. Thus, a reliable and sensitive general phenol analysis could be a useful tool for determination of a relatively large number of pesticides.

A comprehensive comparison of five of the more sensitive methods for phenol determination has been made by Mohler and Jacob (*2*). The 4-aminoantipyrine (4-AAP) method (*3*) was the fastest one, while offering most quickly the greatest precision and accuracy. Moreover, it seems to be the one

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(1) F. A. Gunther and D. E. Ott, *Residue Rev.*, **14**, 12 (1966).
(2) E. F. Mohler, Jr. and L. N. Jacob, ANAL. CHEM., **29**, 1369 (1957).
(3) M. B. Ettinger, C. C. Ruchhoft, and R. J. Lishka, *ibid.*, **23**, 1783 (1951).

generally adopted for water and waste water analysis (*4*), and also is applied rather frequently in other fields—*e.g.*, for determination of some pesticide moieties (*5–7*).

The 4-AAP reaction has several deficiencies, the most significant of which is its discrimination against certain *p*-substituted phenols. Other disadvantages are its sensitivity for pH-variations and the consequent difficulty of finding suitable buffers for the reaction (*5*).

Shortly after the comparison mentioned was made, Hünig and Fritsch (*8*) reported discovery of new azo dyes obtainable by the oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with phenols, reactive methylene compounds, or aromatic amines. Since then, however, little attention has been paid to the possibility of this reaction as a basis for determining phenols. Thus far, the reaction between MBTH and phenols has been utilized in a spot test for phenol derivatives (*9*) and in a manual procedure by Umeda for determination of phenol itself (*10*). Due to the fact that the last-mentioned work is published in Japanese and that no comparison with other phenol methods is made, the method does not seem to have been subject to widespread application. The present paper describes an automated procedure for the determination of phenols. For the purpose of comparison with other methods, usable conditions for performing the reaction manually are also given. The method is based on the reaction (*8*) shown in Figure 1, and utilizes the feature of Umeda (*10*) of performing the coupling reaction in acid medium using ceric ammonium sulfate as oxidant instead of potassium ferricyanide (*8*).

EXPERIMENTAL

Reagents. MANUAL PROCEDURE. 0.05% (w/v) aqueous solution of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH). (Code 1996, K & K Laboratories, Inc., Hollywood, Calif.).

0.2% (w/v) ceric ammonium sulfate in 0.4% (v/v) sulfuric acid $[(NH_4)Ce_4(SO_4)_4.2H_2O$, Code 1569, Allied Chemical Corp., Morristown, N. J.]. Prepare fresh daily.

Buffer solution. Dissolve in the following order, 4.8 grams of NaOH, 2.0 grams of EDTA (disodium salt), and 8.0 grams of boric acid in 500 ml of water. Using this stock solution make a fresh working solution daily by mixing an appropriate amount with an equal volume of ethanol.

AUTOMATIC PROCEDURE. 0.05% (w/v) aqueous solution of MBTH in 1.0% (v/v) of Sterox NJ-detergent solution (Monsanto Co., St. Louis, Mo.). When not in use the MBTH solution may be stored refrigerated for at least one week.

1.0% (w/v) ceric ammonium sulfate in 1.5% (v/v) sulfuric acid.

Buffer solution. Dissolve in the following order, 8.0 grams of NaOH, 2.0 grams of EDTA (disodium salt), and 8.0 grams of boric acid in 1,000 ml of water.

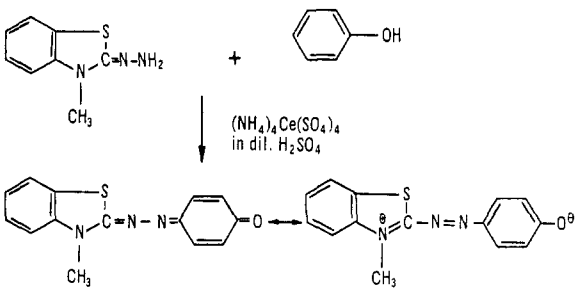

Figure 1. Oxidative coupling reaction; MBTH and phenol

Table I. Molar Absorptivity Values

Compound Name	Molecular weight	λ_{max} (mμ)	Reaction product: Apparent[a] molar absorptivity (× 10^{-3}) Present method	4-AAP method
Phenol	94.1	520	26	14
2-Cresol	108.1	500	24	14
3-Cresol	108.1	510	23	9
4-Cresol	108.1	550	9	...[b]
4-Chlorophenol	128.6	520	22	9
4-Bromophenol	173.0	520	23	11
3-Iodophenol	220.0	530	28	19
3-Aminophenol	109.1	460	19	12
4-Aminophenol	109.1	500	10	...[b]
2-Nitrophenol	139.1	550	14	7
3-Nitrophenol	139.1	520	20	5
4-Nitrophenol	139.1	500	2	...[b]
4-Hydroxybenzoic acid	138.1	525	18	13
4-Methylthiophenol	140.2	520	20	15
2,4-Dichlorophenol	163.0	530	28	22
2,4-Dibromophenol	251.9	530	36	30
2,6-Dibromophenol	251.9	470	23	29
4-Chlororesorcinol	142.6	475	27	15
3-Methyl-4-methylthiophenol	154.2	500	13	16
2,4-Dinitrophenol	184.1	...[b]	...[b]	...[b]
2,4,5-Trichlorophenol	197.5	520	16	23
2,5-Dichloro-4-bromophenol	241.9	535	20	19
2,5-Dichloro-4-iodophenol	288.9	530	20	22
2,6-Diiodo-4-cyanophenol	370.9	...[b]	...[b]	...[b]
2,4′-Dihydroxy benzophenone	214.2	475	23	...[b]
4,4′-Dihydroxy benzophenone	214.2	595	11	...[b]
2-Cyclohexylphenol	176.3	505	24	14
4-Cyclohexylphenol	176.3	540	16	...[b]
2-Phenylphenol	170.2	520	30	18
4-Phenylphenol	170.2	580	13	...[b]
1-Naphthol	144.2	510	11	9
2-Naphthol	144.2	510	19	9

[a] See discussion section.
[b] No reaction.

Phenols. The phenols used in these experiments were of reagent grade, or else recrystallized to constant melting point. A 100-ml stock standard solution at 500 μg/ml of each phenol was made up in water after initial solution in 1 ml of 1*N* NaOH. Working dilutions with water at 5 μg/ml or less were prepared immediately and refrigerated when not in use. Some of the stock solutions needed to be discarded after this first usage due to instability under these relatively high alkaline conditions; even some of the diluted standard solutions should be prepared fresh weekly or oftener.

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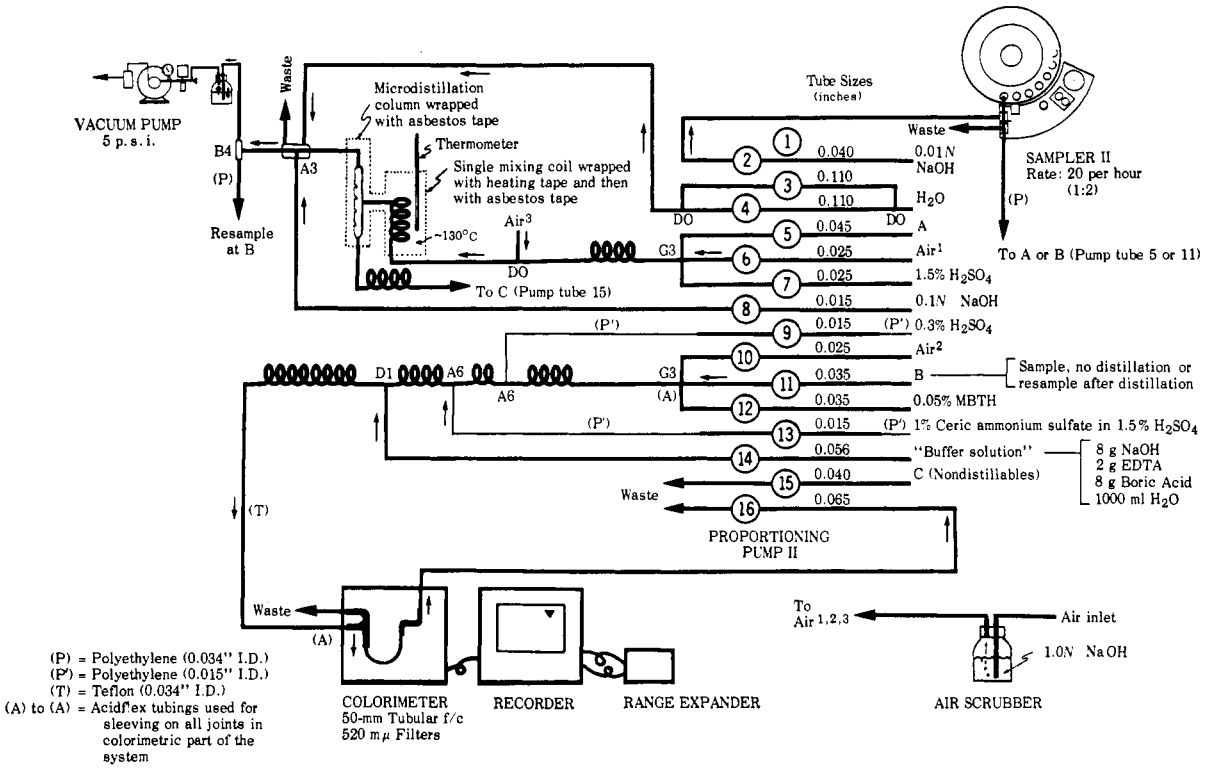

Figure 2. AutoAnalyzer for phenols

Procedures. MANUAL. Mix 1.0 ml of the aqueous test solution containing an appropriate amount of the phenol (derivable from the molar absorptivity values given in Table I, generally about 1 μg minimum) with 1.0 ml of MBTH solution in a test tube. After 5 minutes add 1.0 ml of 0.2% ceric ammonium sulfate solution and shake the mixture. Wait another 5 minutes and add 2.0 ml of the buffer solution. Shake well and read the absorbance after 15 minutes at the maximum absorbance wavelength (see Table I) against water as reference using 1-cm cells. Subtract the absorbance value of a reagent blank.

AUTOMATIC. All equipment used including the microdistillation column was obtained from the Technicon Corp., Tarrytown, N. Y., with the exception of the heater tape ($^1/_2$″ × 2′, 96 W) and its associated variable resistor. This heater tape is turned on only after starting the proportioning pump and turned off only after shutting down the pump; otherwise, superheating will result.

The flow diagram of the method is shown in Figure 2. Because several of the phenols tested are adsorbed to the Tygon transmission tubing (and even stronger to Solvaflex tubing), all connections should as far as possible be glass-to-glass with Acidflex sleevings. To decrease adsorbency of phenols to the inner wall of the sample pump tubing, add one drop of 1*N* NaOH to each 2-ml sample cup just before the sampling is started. Some phenols—*e.g.*, 4-aminophenol—are too unstable under alkaline conditions, in which case the sodium hydroxide addition should be omitted. Run the samples through at a rate of 20 per hour using a cam in the sampler giving a sample-to-wash ratio of 1:2 with pickup by means of a 0.034″ i.d. stainless steel probe.

If the distillation step is used, the vacuum is held constant at 5 psi, but the flow rate of air through the distillation coil is adjusted by means of a pinch clamp around the Tygon tubing connected to the top of the B4 glass fitting to ensure a constant liquid level in the lower part of that fitting. Some adjustment of temperature may also be required when first setting up the instrument; the recommended approximate 130 °C value indicated in Figure 2 cannot be considered absolute because the positioning of the thermometer is arbitrary. The distillation step must of course be omitted if the phenol in question is not steam distillable (see bottom of Figure 3).

RESULTS AND DISCUSSION

Development of Method. The initial experiments with the MBTH–phenol reaction were performed under alkaline conditions with potassium ferricyanide as oxidant as described by Hünig and Fritsch (*8*). While this was satisfactory for some phenols, the yield of dye was generally low. In some instances improvements were obtained by changing the oxidant to ammonium persulfate (*11*), which reacts more slowly, however. A remarkable increase in yield of dye was experienced by using ceric ammonium sulfate and performing the coupling reaction under acid conditions (*10*).

The ceric ammonium sulfate solution should contain the amount of sulfuric acid required to obtain optimal acidity for the coupling reaction, because ceric hydroxide will start to precipitate at pH values above 0.8 (*12*). In a preliminary automated system, problems were experienced due to adsorption of colored material to the last two mixing coils and the flowcell, eventually causing an increasing recorder baseline. The annoyance was traced to the oxidant introduction area where the slightly alkaline sample solution was causing an irreversible precipitation of ceric hydroxide before sufficient mixing had taken place. By mixing the sample–MBTH stream with sufficient acid in a half mixing coil inserted before the oxidation step, the problem was overcome.

When the coupling reaction has taken place, alkaline conditions advantageously decrease the background color and

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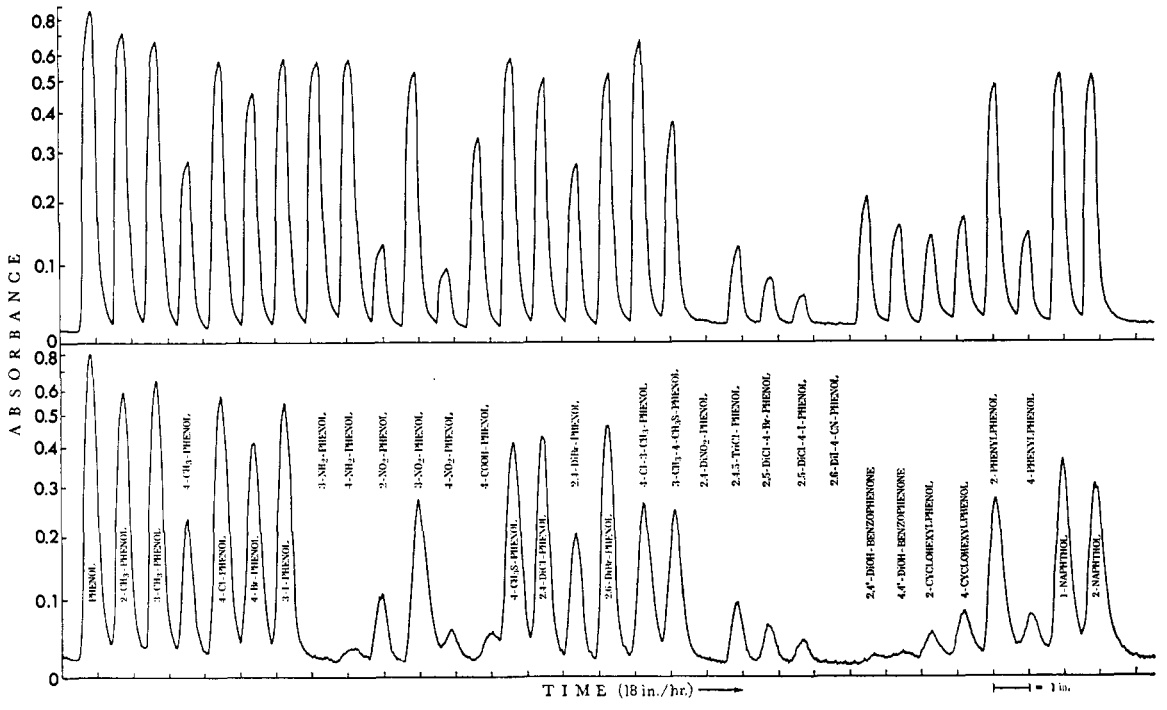

Figure 3. Typical chart records from the AutoAnalysis of various selected phenols (top—without steam distillation; bottom—same series of phenols with use of automated steam distillation)

increase the absorbance values of the dyes. It was found possible to prevent the precipitation of ceric hydroxide upon this change of pH by adding EDTA to the alkali solution. Addition of ethanol to the alkaline dye solution had a stabilizing effect on some of the colors; the buffer solution used for the manual procedure therefore contains ethanol. Under these conditions the absorbances of most of the dyes were stable over 90 minutes. In the automatic procedure all steps are so precisely timed that the ethanol addition was found to be superfluous.

The reagents for the manual and the automatic procedures are slightly different also in other respects. Thus, the 1.0% ceric solution giving optimal absorbance values in the automated procedure gave inconsistent results when used in the manual way. This problem was overcome by using a larger volume of a less concentrated oxidant solution. A possible explanation for this phenomenon is that the bulk addition of a relatively concentrated oxidant solution could cause an oxidative breakdown of the MBTH reagent.

The steam distillation step was originally performed by use of the commercially available Technicon microdistillation unit. However, the relatively long and large diameter Teflon (Du Pont) coil used in the heating bath of the unit causes considerable diffusion resulting in much broader and lower recorder peaks.

Capability of Method. Figure 3, at the top, shows a typical recording obtained with a series of 32 different phenols using the automated procedure with omission of the steam distillation step. Each peak represents the response from a sample containing 5 μg/ml of the respective phenol. All colorimetric measurements were made without range expansion using a 520-mμ filter. A situation where such a complex series of different phenols has to be analyzed will obviously rarely, if ever, occur. The tracing is shown merely to indicate the general applicability and the low detectability limit of the method. The corresponding results with the automatic steam distillation step included are shown in the bottom of Figure 3.

The effects of varying the concentration of the MBTH solution, the concentration and acidity of the oxidant, and the alkalinity of the final color solution of all the phenols have been investigated for the automatic procedure. A set of conditions best for one phenol may not be best for another; thus the finalized procedure with reaction conditions as described is a compromise procedure selected to determine as many of the chosen phenols as possible.

The addition of relatively high concentrations of a detergent to the MBTH reagent is required especially to obtain good flow characteristics when low minimum detectability is desired. The detergents generally used for this purpose in automated analytical systems (Brij 35 and Levor IV), gave less satisfactory results by giving background color and/or precipitation with MBTH; in this procedure Sterox NJ was the best of several detergents tested.

Table I shows the wavelength of maximum absorbance for the 32 different phenols tested with the manual procedure and with a Beckman Model DB spectrophotometer. The colors obtained have λ_{max} values ranging from 460 to 595 mμ and are mostly red to violet. When analyzing for a specific phenol with the AutoAnalyzer, a colorimeter filter corresponding to the wavelength of a maximum absorbance, therefore, should of course be used to increase the minimum detectability of the method.

The figures found under the heading "apparent molar absorptivity" in Table I are not the absolute molar absorptivity values. Rather they are calculated values assuming 100% conversion of the appropriate phenol to its respective dye. Because this assumption rarely holds true, the absolute molar absorptivity will usually be considerably higher than shown in the table—*e.g.*, for phenol, absolute values of 64,000 in chloroform (*13*) and 45,000 in dimethyl formamide (*8*) have been reported. The apparent molar absorptivity figures are based upon absorbance values read at the wavelength of

(13) Siegfried Hünig and Heinz Balli, *Ann. Chem.*, **628**, 56 (1959).

maximum absorbance using the manual procedure. The standard phenol solutions used corresponded to a final concentration of 1 μg/ml, calculated as unreacted phenol.

For comparison, Table I also contains similarly calculated apparent molar absorptivity values obtained in the same way with the 4-AAP method. The analyses were performed manually according to Method C of Standard Methods (*4*) which generally gave higher results than did the method of Ettinger, Ruchhoft, and Lishka (*3*). As indicated by the Table, the MBTH method not only is more universal in reactivity to phenols but also shows generally higher molar absorptivities. Only two of the phenols tested, 2,4-dinitrophenol and 2,6-diiodo-4-cyanophenol, give completely negative results. The former has two strong *meta*-directing substituents in two of the three possible coupling positions, and in the latter compound all positions are occupied. It is interesting to note that some reaction takes place with 4-nitrophenol; however, the apparent molar absorptivity usually will be too low for practical analytical purposes.

It should be noted that 20 of the 32 phenols tested have a substituent in the *p*-position; they were purposely selected to give the method a more critical evaluation, because phenols with free *p*-positions were considered more likely to react.

The precision of the automated procedure has been studied with and without inclusion of the steam distillation step. Repeated analyses performed without steam distillation over six consecutive days on a standard solution containing 5 μg/ml of phenol, showed a relative standard deviation of 3.3%. This figure incorporates, among other factors, variations normally expected from small inconsistencies from day to day in pumping flow rates of samples and reagents, differences in age of reagents, and possible breakdown of the phenol during storage of its standard solution. A similar precision study of seven of the intermediate reactive phenols gave a maximum standard deviation value of 8.3% for 4-aminophenol and an average relative standard deviation for all these eight phenols of 5.1%. When the sampling was made during one single day by running 10 samples intermixed with the same number of a 5× less concentrated standard solution, the relative standard deviation was found to be 0.8% and 1.3% for the high and the low concentrations, respectively. The corresponding values obtained with inclusion of the steam distillation step were 1.5% and 2.0%, respectively. These relative standard deviation figures relate to analyses performed without a range expander connected to the recorder. For determinations in the parts per billion (ppb) concentrations, however, range expansion is required. Figure 4 shows the recorder tracing of a series of standard solutions of phenol in the 10–200 ppb range analyzed at 10× range expansion with the steam distillation step included. It is apparent from the curve that the precision is satisfactory also for these low concentrations. Consecutive analyses of 10 samples at 150 ppb gave a relative standard deviation of 7.6%. The lower detectability limit for phenol is about 10 ppb. For the other phenols tested the lowest concentrations detectable by the method are higher, but approximately half of them may still be determined at concentrations below 100 ppb.

Interference from Other Compounds. MBTH reagent has been used in analyses for some compounds other than phenols: for certain aliphatic aldehydes (*14*), for carbazoles, azo dyes, stilbenes, and Schiff bases (*15*), and for aromatic amines and

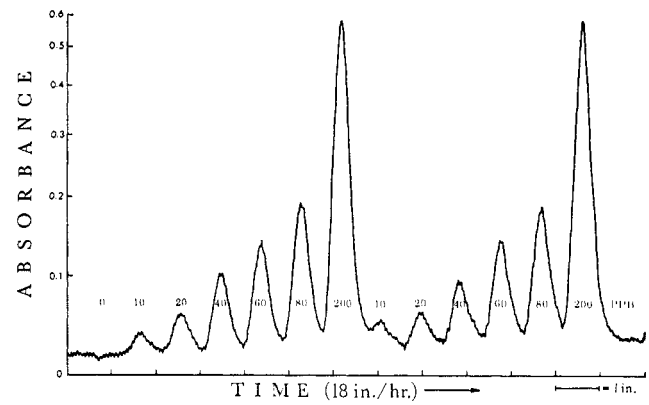

Figure 4. Parts per billion concentration solutions of phenol AutoAnalyzed with use of automated steam distillation

imino heteroaromatic compounds (*16*). Because acid coupling conditions have been chosen for the present method, interference can be expected from aliphatic aldehydes and from aromatic amines. Because very high absolute molar absorptivities have been reported for the reaction products of such compounds, they will have to be removed before phenol determinations even though their λ_{max} values usually will be found in a part of the spectrum different from those of the phenol dyes. When the colorimetric part of the procedure is preceded by the steam distillation step, aromatic amines will not interfere. As seen in the bottom of Figure 3, even phenols containing amino groups (peaks 8 and 9 from the left) do not distill from these relatively strong acid conditions. It should be possible, however, to use the steam distillation step as a cleanup procedure also for aminophenols by performing the distillation from a solution with a pH value close to the isoelectric point.

Because ethanol usually contains acetaldehyde as an impurity, it should not be present during the oxidative coupling step. Acetone reacts with the MBTH reagent and stops the coupling reaction and should, therefore, likewise be excluded.

Just as some improvements of this general procedure probably can be expected by variation of one or more of the reaction conditions if applied for the analysis of a specific phenol, the interference from other compounds will also have to be considered in each particular case. When used for monitoring water supplies for phenols, the steam distillation step should usually provide the only cleanup necessary, whereas analyses for pesticide residues in foodstuffs will usually require additional sample treatment to remove other interfering phenolic compounds.

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